

December 16, 2004

William Stokes, D.V.M.
Director, NICEATM
National Toxicology Program
PO Box 12233, MD EC-17
Research Triangle Park, NC

Dear Dr. Stokes:

This public comment is delivered in response to Federal Register Notice volume 69, Number 212, Pages 64081-64082. It addresses Background Review Document (BRD), "Current Status of In Vitro Methods for Identifying Ocular Corrosives and Severe Irritants: the Bovine Opacity and Permeability (BCOP) Test Method", November 1, 2004.

Since 1997 Mr. Philip Casterton (until recently at Alticor Corp. Ada, MI; formerly Amway Corp.), my students at Calvin College and I have been working on a project the goal of which is to evaluate and improve the BCOP assay. As a result of this work we have made some suggestions for modification of the assay and have developed a holder that does not damage the cornea. We have published 5 papers on our work and have made presentations at 9 national and international meetings. These included invited presentations that I made at ARVO, as well as the NIH conference on alternative toxicological methods in 2000 (see publication 4, reprint enclosed) and the workshop sponsored by the Institute for In Vitro Science in June 2003, both of which you attended.

Our major contribution to research on the BCOP assay is the development of an improved corneal holder for the assay that does not damage the edges of the cornea and maintains the normal shape and curvature of the bovine cornea, as compared to the standard holder currently used in the BCOP assay. The BDR concerning the BCOP assay accurately summarizes our work that was published in references 1, 2 and 3 below, but does so without comment. I am enclosing a reprint of our most recent publication (5) in which we show that use of the improved corneal holder for the BCOP assay yields lower and less variable permeability values than the standard corneal holder.

My interest in the BCOP assay arose from my background as a corneal physiologist with training in the laboratory of Henry Edelhauser, Ph.D., a member of the ICCVAM expert panel charged with evaluating the BCOP assay. When I first became familiar with the BCOP assay I was immediately concerned about the

degree of edge damage to the cornea caused by the corneal holders since corneal physiologists are fastidious about avoiding all damage to corneas that are mounted for in vitro measurements of parameters such as permeability, electrical activity, endothelial pump function and hydration levels. In reviewing publications about the BCOP assay I was surprised about the relative lack of attention to the corneal literature and the fact that validation studies did not interpret the opacity and permeability data in terms of known mechanisms affecting corneal transparency and barrier function. I evaluated the BCOP assay in a report that was submitted to the International Life Sciences Institute (ILSI) in response to an ILSI panel meeting on Alternatives to Animal Testing in which I participated on September 29-30, 1996. A copy of that report is enclosed. This evaluation of the BCOP assay led to our experimental work on the test.

In our first study of the BCOP assay (1) we used the standard holder and focused on understanding the opacity measurement in terms of corneal hydration, but the morphologic and histological work that was included in this study made us aware of the degree of corneal damage caused by the corneal holders. In our second publication (2) we attempted to add evaluation of the corneal endothelium to the BCOP assay, but the degree of damage to the endothelium caused by simply mounting the cornea in the standard holder made our data unreliable. We showed that clamping the large, oval-shaped 24 x 30 mm bovine cornea between the flat surfaces of the holder and forcing it to "conform" to the circular 17 mm diameter opening in the holders caused wrinkling of the cornea. Damage to the endothelium corresponded directly to the wrinkles. Because of the essential role of the endothelium in maintaining corneal transparency it was evident that a new corneal holder that does not damage the corneal endothelium was needed if a valid BCOP assay was to be developed.

With assistance from Mr. Dennis Kool, an engineer at Alticor Corp., we have developed a corneal holder that maintains the normal shape of the cornea, does not cause edge damage, does not damage the endothelium, and yields significantly lower control permeability than the standard holder (3-5). It is our opinion that the BCOP assay should be conducted using this modified holder since reliable toxicological data can only be obtained from healthy tissue. All biological experiments must be conducted under optimal conditions. Animal studies are conducted using healthy animals. Cell culture (including toxicological methods, such as the neutral red release assay) is conducted using uncontaminated cells grown in optimal medium. In biochemical and molecular biology research all reagents must be of the highest quality. In the same way, assays using isolated organs or tissues must be conducted using undamaged specimens that are initially in optimal condition. This is not the case with the BCOP assay as it is currently conducted since, as we have shown, the assay begins with a severely damaged cornea that cannot be considered to be anatomically or physiologically normal.

Because the BCOP assay as currently conducted does not meet accepted physiologic standards for studies of isolated corneas I believe that it should not and cannot be validated as alternative toxicological method. Using a flawed alternative method and attempting to validate it with respect to the Draize test, which itself may be criticized on scientific grounds, is not predictive of success in reaching our goal of developing a valid method for testing ocular irritants. The holder currently used must be discarded, and a new data base must be

established using methods that do not damage the cornea independently of effects of test materials. Consideration should also be given to using porcine corneas which may prove to be a better model for the human cornea. More importantly, an optimized BCOP type method should ideally be validated in a way that we may be confident that the data obtained are predictive of human response to irritants.

I recommend that the expert panel consider ways in which funding can be obtained so that adequate numbers of the holders that we have developed can be produced, permitting large scale, multi-laboratory validation studies of a modified BCOP assay to be conducted. I would be eager to work with you in meeting these goals.

Sincerely,

John L. Ubels, Ph.D.
Professor of Biology
Calvin College
Grand Rapids, MI

Adjunct Professor of Ophthalmology
Wayne State University School of Medicine
Detroit, MI

Publications

1. Ubels J.L., Erickson A.M., Zylstra U., Kruielen C.D. and Casterton P.L. (1998) The effect of corneal hydration on opacity in the bovine cornea opacity and permeability assay. *Journal of Toxicology – Cutaneous and Ocular Toxicology* 17, 197-220.
2. Ubels J.L., Pruis R.M., Sybesma J.T. and Casterton P.L. (2000) Corneal opacity, hydration, and endothelial morphology in the bovine cornea opacity and permeability assay using reduced treatment times. *Toxicology in Vitro* 14, 379-386.
3. Ubels J.L., Paauw J.D., Casterton P.L., Kool D.J. (2002) A redesigned corneal holder for the bovine cornea opacity and permeability assay that maintains normal corneal morphology. *Toxicology in Vitro*. 16, 621-628.
4. Ubels J.L., Paauw J.D., Casterton P.L. (2003) Evaluation and refinement of the bovine cornea opacity and permeability assay. In: *Alternative Toxicologic Methods*. Katz S., Salem H. (eds) CRC Press LCC, Boca Raton, FL pp. 107-113.
5. Ubels J.L., Ditlev J.A., Clousing D.P., Casterton P.L. (2004) Corneal permeability in a redesigned corneal holder for the bovine cornea opacity and permeability assay. *Toxicology in Vitro*. 18, 853-857.